The Respiratory Tract as a Portal of Entry for Toxic Particles

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Our ambient, external environment poses a constant threat to the life and health of cells that make up our body. The external environment is cold, dry, septic (putrefactive), toxic, and widely variable in chemical composition, salinity, and acidity. In contrast, our internal environment is represented by the fluid that surrounds our cells and keeps them alive. The internal environment is warm, wet, sterile, and nontoxic, and has an ionic-chemical composition that is closely regulated by homeostatic processes. The internal and external environments confront each other across epithelial barriers, comprising primarily the skin, the gastrointestinal tract, and the respiratory system. These barriers are differentially susceptible to attack, and the route by which a toxic insult enters the body can determine its effectiveness. Pure water in the lungs or pure air in the circulating blood are more life threatening than polluted air in the lungs or alcohol in the circulation.

The purpose of this chapter is to contrast the three major portals of entry, with particular emphasis on the lungs and the entry of inhaled particles. Our lung surfaces, due to their primary function of gas exchange, come into intimate contact with irritating gases and airborne particles. The same thinness and extensive area that qualify this air-blood barrier for the rapid exchange of oxygen and carbon dioxide reduce its effectiveness as a barrier to inhaled microorganisms, toxic particles, and noxious gases [1]. Inhalation of these agents may initiate or aggravate lung disease. In order to assess adequately the risks of inhaled-particle exposure, we need to characterize the fate of particles entering the respiratory tract.

ANATOMICAL CHARACTERISTICS

Some of the anatomical differences between the skin, gastrointenstinal tract, and the respiratory system are summarized in Table 1. The skin envelops the outside of the body and is a mechanically strong epithelium, with many complex specializations such as hairs, nails, pigmentation, and glands. The total weight of skin (dermis plus epidermis) is about 12 kg in a 70 kg human. The gastrointestinal tract is a long tube topologically continuous with the skin at both ends and exhibits absorptive and surface area specializations along its length; its total weight is about 7 kg. The respiratory system begins at the mouth as a single pathway that repetitively bifurcates into a complex branched system of tubes, which terminate in blind-ended sacs, the alveoli. The lungs, being air-filled, contribute only about 1% to body weight, or 0.8 kg [2]. The skin, gastrointestinal tract, and lungs have important elastic and smooth muscle components, but the lungs are unique in that an important function, that is, expiration, is crucially dependent on its elastic properties alone.

In the context of route-to-route extrapolation, the significant anatomical characteristics of these barriers are their surface area and thickness (Table 1). The barrier function of the skin is evident with its much smaller surface area (1.8 m²) and considerably greater thickness (100 to 1000 μm) when compared to the other two epithelial barriers. In the gastrointestinal tract, surface area is not limited to that of a 10-m long tube, but is augmented by intestinal folds, villi, and microvilli, to achieve a surface area equivalent to about a doubles tennis court (200 m²). Most of the gastrointestinal tract absorptive epithelium is simple columnar so that the distance from lumen to blood is approximately 8 to 12 μm. In the lungs, a large surface area (a singles tennis court, 140 m²) is achieved by repetitive branching (about 16 generations) so that the initial tube, the trachea, is connected to 300 million alveoli [3]. The gas-exchange epithelium is simple squamous, giving a very short distance (0.2 to 0.4 μm) between the air and blood.

FUNCTIONAL DIFFERENCES

The functions of the skin are primarily those of a barrier, that is, to prevent entry of microorganisms and other environmental agents and to prevent water and heat loss. The gastrointestinal tract has absorptive capacities that are both active (i.e., can work against a concentration gradient) and well regulated (i.e., degree of absorption can be modified). In addition, bacteria that thrive in the gastrointestinal tract are prevented from entering the circulation. The respiratory epithelium exchanges oxygen and carbon dioxide, both of which diffuse passively down concentration gradients. The air-liquid interface of the lungs is an additional unique property of this barrier. Like the other two barriers, inhaled pathogens must be prevented from reaching the blood. However, the lungs also have other "functions", namely, vocalization, coughing, sneezing, and straining in defecation [4].

Some of the functional characteristics relevant to route-to-route extrapolation are shown in Table 2. The quantity of exposure is dramatically different among the several routes. On a daily basis, the mass of air we inhale (approximately 24 kg) exceeds by far the mass of material entering daily into our gastrointestinal tract (approximately 2 kg). There are also important blood flow differences. The lungs always receive the total cardiac output. The gastrointestinal tract and the skin receive only a (variable) fraction of total blood flow. At rest, the gastrointestinal tract and skin receive about 25 and 10% of cadiac output, respectively. During exercise total cardiac output may triple, but the gastrointestinal tract percentage falls to 3%, and the skin percentage rises slightly to about 12% [2]. Flow in the gastrointestinal tract is generally unidirectional, proceeding from one orifice to the other. Flow in the lungs is tidal; that is, airflow reverses on a periodic basis, and air moves in and out of a single orifice.

The time scale for throughput is another relevant consideration when assessing functional differences. Breathing is an act that must be continuous on a minute-by-minute basis, whereas the intervals

Table 1. Anatomy of epithelial barriers.

Interface with Environment	Area (m²)	Thickness from Environment to Blood (μm)	Organ Weight (kg)
Skin	1.8	100-1000	12
Gastrointestinal	200	8-12	7
Lungs	140	0.2-0.4	0.8

Table 2. Epithelial barrier dynamics.

Interface with Environment	Basal Blood Flow (L/min)	Cell Turnover (days)	Basal Exposure Rate
Skin	0.5	12	variable
Gastrointestinal	1.4	3	2 kg/day
Lungs	5.8	28	24 kg air/day

between food and water intake can be much longer. This means that our choice of the air we breathe is less voluntary than of the food and water that we ingest. In fact, world-record breath-holding time (13 min, 42.5 sec) is much shorter than world-record fast duration (382 days) [5]. Finally, the dynamic range of breathing between sleep and heavy exercise can cover a factor of about 30 in minute ventilation, so that delivery of potentially polluted air to respiratory surfaces is dependent on state of exercise [4].

INHALATION OF AIRBORNE PARTICLES

One hundred years ago, in 1882, John Tyndall published his essay "Floating-Matter of the Air in Relation to Putrefaction and Infection." Using the light-scattering instrument that bears his name, Tyndall showed that the air we exhale is less dusty than the air we inhale, thus demonstrating that the lungs act as a filter for airborne particles. The three main factors acting to bring inhaled particles in contact with lung surfaces are (1) settling under the influence of gravity; (2) particle inertia, which carries particles straight when airflow turns; and (3) particle Brownian diffusion from random gas collisions [6, 7]. The relative effect of particle settling versus diffusion can be appreciated by examining Table 3, which shows the relative amount of distance traveled by unit density particles of different size. For example, in 1 sec a 2-μm diameter particle diffuses a root-mean square distance of 8.8 μm, whereas during the same time it falls 125 μm, so that settling is the dominant influence in moving the particle toward lung surfaces. On the other hand, a particle 0.1-μm diameter diffuses a distance of 64 μm in 1 sec, but falls only 0.81 μm; thus Brownian motion is a more important deposition mechanism [8].

In addition to particle characteristics, aerodynamics of respiration and anatomy of the airspaces influence particle deposition. The nose acts as a prefilter, capturing very large particles (5 to 10 μm). Large particles are also susceptible to inertial impaction in the airways where flow is high and air streamlines change directions frequently. Particles that penetrate to the small bronchiolar and alveolar region can be collected rapidly by settling and diffusion. Total collection efficiency for the lung is lowest in the particle size range around 0.5 μm, because these particles do not settle very rapidly, yet they are too large to diffuse effectively (cf. Table 3, the sum of Brownian displacement in 1 sec plus distance fallen in 1 sec is least for 0.5-μm particles). Aerodynamics of respiration also influences particle delivery and deposition. Minute ventilation can vary from a low of about 5 L/min at rest to a high of about 140 L/min, which is maximum voluntary ventilation. Delivery of particles to the lungs varies in direct proportion

Table 3. Brownian diffusion (root-mean-square) in 1 sec compared with distance fallen in 1 sec for unit density particles of different diameter.a

	Particle Diameter (μm)	Diffusion in 1 sec (μm)	Distance Fallen in 1 sec (μm)
Settling greater in 1 sec	50	1.7	70,000
	20	2.7	11,500
	10	3.8	2,900
	5	5.5	740
	2	8.8	125
	1	13.0	33
Diffusion greater in 1 sec	0.5	20	9.5
	0.2	37	2.1
	0.1	64	0.81
	0.05	120	0.35
	0.02	290	0.013
	0.01	570	0.0063

a Temperature: 37 °C; gas viscosity: 1.9×10^{-5} Pa-s; appropriate correction factors are applied for motion outside the range of validity of Stokes Law.

to minute ventilation. The ventilatory pattern can modify deposition. Slow, deep breathing delivers more particles distally than rapid, shallow breathing. Total deposition is greater with slow, deep breathing, and is more uniformly distributed than with rapid, shallow breathing [9, 10].

Dose to the respiratory tract from inhaled particles is proportional to particle retention, and integrated particle retention is derived from the balance of the two processes: deposition and clearance. The mechanics of these processes are presently not understood well enough to calculate retention with confidence from a priori structure and function data. Comparison of experimental morphometric, physiologic, and cellular characteristics of the respiratory tract among different mammalian species allows some insight into mechanisms that may be important when using animal data to evaluate the human respiratory tract as a route of toxic particle entry [11]. Examples of such parameters include ventilation per unit surface area, average lung airspace size, mucociliary clearance rate, and pulmonary macrophage number per unit lung surface area (Table 4) [12, 13].

TABLE 4. Lung and alveolar macrophage parameters as they may relate to *in vivo* particle uptake.

	Mammalian Species						
	Mouse	Hamster	Rat	Guinea Pig	Rabbit	Dog	Human
Avg. body wt. (g)	42	122	380	430	2600	16,000	74,000
V_L (mL)	1.45	3.9	10.9	13	112	1320	4340
S_A (m²)	0.125	0.28	0.66	0.91	3.3	52	143
Alveolar diam. (μm)	47	60	70	65	88	126	219
Calculated # of alveoli (millions)	18	25	43	69	135	1040	950
Average # of lavagable AMs per animal (millions)[a]	0.67	4.7	4.9	3.2	30	3800	6400
Calculated AMs per alveolus	0.037	0.19	0.11	0.046	0.22	3.7	6.8
Area patrolled by each AM (μm²)	190,000	60,000	140,000	280,000	110,000	13,400	22,000
In vivo particle uptake by AM ($T_\frac{1}{2}$, hours)	7.1	0.8	4.2		3.2	Correlation coefficient with area = $r^2 = 0.99$	

[a] AM = Alveolar macrophage

DEFENSE MECHANISMS FOR THE THREE ROUTES

Defense against penetration of the skin relies primarily on the mechanical strength of the cornified layer skin in addition to the underlying stratified squamous cells, which are linked to each other by tight junctions. The sebaceous glands, which secrete an oily/waxy layer coating the skin, are an additional line of defense. However, even though the skin is resistant to aqueous toxins, ionic, organic, and lipid-soluble agents can penetrate. Carbon tetrachloride (CCl_4), organophosphate pesticides, and coal tar pitch volatiles (CPTV) are examples of toxic substances that can cross the skin and cause deleterious effects in the liver (CCl_4) or nervous system (organophosphates), or can cause skin (scrotal) cancer (CTPV). Finally, skin cells slough off with a time constant of 12 days so that toxins in the outer layers can be removed [14].

The gastrointestinal tract has several first-line defenses: vomiting, the acidic environment of the stomach, and the proteolytic environment of the small intestine. The gut epithelium comprises metabolically active columnar cells, and uptake from the gut is selective to some degree. Furthermore, the constant throughput of the gastrointestinal tract assures that substances will remain in contact with the epithelium for only a limited amount of time. The turnover time of the gut epithelium is very rapid (about three days), and damaged, leaky cells are rapidly sloughed off and replaced by vigorous counterparts. Finally, because blood outflow from the gastrointestinal tract goes directly to the liver, toxins can be potentially deactivated before reaching the general circulation.

The first line of defense of the respiratory tract are the cough and sneeze reflexes. In the nose, fine hairs filter out large particles. In the major airways, a mucus coating serves two defense functions. First, if particles settle on the mucus, the mouthward transport driven by the underlying cilia ensures that the particles are moved out of the lung and into the gastrointestinal tract, where they can be eliminated from the body. Second, for toxic, reactive gases, such as ozone, the mucus forms a protective layer that reacts with these agents and thereby protects the epithelium underneath. Surfactant in the alveoli may serve this role to a lesser degree due to its limited thickness. The alveolar epithelial cells provide less protection than those in the gut because they are thinner and less metabolically active. Moreover, the turnover time of the alveolar epithelium is about 28 days so that damage is not as easily repaired [15, 16].

The alveolar surfaces are, however, protected by the pulmonary macrophage, a wandering, phagocytic cell that has remarkable properties in terms of recognizing, ingesting, and deactivating bacteria and particles [16, 17]. The phagocytic process not only exposes the particles (or pathogens) to lysosomal proteolytic enzymes, but also provides a transport mechanism whereby particles can leave the lungs. That is, an inhaled and deposited particle may of itself be completely immobile on the lung surfaces and thus fail to leave the lung over long periods of time. However, ingestion by a macrophage imparts the cell's mobility to the particle, and since the cell may ultimately translocate to the mucus carpet, this route of mechanical clearance now becomes available to the particle. Ingestion by the macrophage also helps prevent particle penetration through the epithelium into interstitial and lymphatic compartments where clearance likely proceeds by solubilization alone.

The time scales of particle clearance are dramatically different between lung and gastrointestinal tract. Due to the continuous

motility of the gastrointestinal contents, ingested material generally passes out of the body in 24 h. Although this time constant is similar to the time needed for material caught in the mucus to be transported out of the lungs, clearance of insoluble particles from the alveolar lung region takes much longer, with half times in the range of six months to several years [18, 19].

The ability of the lung macrophage to clear insoluble particles depends on several factors: (1) the intrinsic ability of the macrophage to phagocytize particles, (2) the motile ability of the macrophage (which may be inhibited by increasing particle load) [20], (3) the amount of lung surface area patrolled by each macrophage, and (4) the average distance between the site of particle phagocytosis and the most distal point to which the mucociliary escalator extends. Intrinsic differences in phagocytic or motile ability among macrophages of different species have not been described, but if the number of macrophages lavaged from the lungs is an indication of the quantity of resident alveolar macrophages, then it would appear that there are systematic differences between the number of macrophages per alveolus and average lung surface area per lung macrophage. These comparisons are shown on Table 4. The number of lung macrophages recovered can be increased by "vigorous" lavage, but since this procedure has been applied extensively only in the rat, the figures used for lavagable alveolar macrophages apply to a more widely-used, gentler procedure. The calculations suggest that macrophages from the mouse and guinea pig must cover a larger surface area and phagocytosis of randomly deposited particles probably proceeds more slowly. In the hamster, dog, and human there are more macrophages per unit surface area, and thus, particles are likely reached sooner. For those species in which *in vivo* colloidal gold particle uptake has been studied, there is good correlation between halftime of gold particle uptake and the area patrolled per macrophage.

SUMMARY

With respect to the integrity of the various epithelial barriers, the respiratory tract seems to be the most susceptible to being breached. Various anatomical and functional characteristics of the lungs contribute to their being a major route of entry of pollutants into the body. The surface area of the lungs is comparable to the gastrointestinal tract, but the thickness of the epithelium is considerably less. The respiratory system has the greatest total mass of environmental media presented to it each day. The blood circulation through the respiratory system is greater than that of the gastrointestinal tract. Clearance of distally deposited material from the respiratory system is more complex and with a longer time constant than in the case of the gut. Finally, repair of epithelial injury is likely not as rapid as in the gut. In light of these considerations, it is surprising that legislation which seeks to protect us from carcinogens (U.S. Food and Drug Administration, Delaney Amendment) is more concerned about the presence of carcinogens in food products than carcinogens present in inhaled consumer products [21].

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